【ABSTRACT】

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The present invention relates to a high density CNT film prepared by laminating a carbon nanotube (CNT) by chemical bonding on a substrate having amine groups exposed and a CNT-biochip comprising bio-receptor bonded to the surface of the CNT film using the functional groups exposed on its surface.

According to the present invention, it is possible to fabricate a CNT-biochip by modifying carboxyl group exposed on a CNT film which is fixed on a substrate with high density with various chemical functional groups, followed binding various bio-receptors by chemical or physicochemical bonding, in which the bio-receptors have a functional group capable of binding to the chemical functional group.

【REPRESENTATIVE DRAWING】

FIG. 1a

【KEY WORDS】

carbon nanotube (CNT), film, receptor, biochip, carboxyl groups, modification

【SPECIFICATION】

【TITLE】

Carbon Nanotube Film with High Surface Density and A Biochip Using the Same

【BRIEF DESCRIPTION OF THE DRAWINGS】

FIG. 1 is a schematic view of the process for preparing a high density CNT film by laminating CNT having exposed carboxyl groups by amide bond on a substrate having exposed amine groups.

FIG. 2 shows the process for fabricating a CNT-DNA chip by binding DNA having amino groups to a CNT film having carboxyl groups exposed, followed performing the DNA hybridization reaction using the CNT-DNA chip.

FIG. 3 shows the process for fabricating a CNT-DNA chip prepared by modifying a CNT film having carboxyl groups exposed with amine groups and binding DNA having carboxyl group as the terminal group thereto, and performing the DNA hybridization reaction using the CNT-DNA chip.

FIG. 4 is an XPS spectrum for phosphorous of the surface of the CNT film having chemically bonded DNA.

FIG. 5(a) shows a fluorescent image of the CNT film of high density, before binding of DNA, FIG. 5(b) shows the result of the fluorescence detection upon hybridization with DNA, after binding DNA having amino group as the terminal group thereto to the CNT film, (1) shows the result of the fluorescence detection upon hybridization with complementary DNA and (2) shows the result of the fluorescence detection upon hybridization with non-complementary DNA.

FIG. 6(a) shows the result of the fluorescence detection upon hybridization with complementary DNA, after binding DNA having carboxyl group as the

terminal group thereto to the CNT film modified with amine groups, and (b) shows the result of the fluorescence detection upon hybridization with non-complementary DNA.

【DETAILED DESCRIPTION OF THE INVENTION】

【OBJECT OF THE INVENTION】

【FIELD OF THE INVENTION AND BACKGROUND ART】

The present invention relates to a carbon nanotube (CNT) film having carboxyl group exposed on its surface and a biochip comprising bio-receptors chemically or physicochemically bonded to a the above CNT film surface, in which the bio-receptors are capable of binding to a target biomaterial.

Carbon nanotube (CNT) is an allotrope of carbon, which abundantly exists on the earth. They are a tubular material where a carbon atom is connected with other carbons in the form of a hexagonal honeycomb structure. Their diameter is in the range of nanometer ($1/10^9$ meter). CNT is known to have excellent mechanical properties, electrical selectivity, field emission properties and highly efficient hydrogen storage properties and be new and almost defect-free of all the existing materials.

Because of their properties of excellent structural rigidity, chemical stability, ability to act as either a conductor or semiconductor and a large ratio of length compared to diameter, CNT exhibits great applicability as a basic material of flat panel displays, transistors, energy reservoirs, etc., and as various electron devices with nanosize.

In order to apply such properties more diversely, the single-wall CNT has been cut into fine pieces using strong acid. The CNT pieces have mainly the -COOH chemical functional groups at a part of the cut ends and side walls. The properties of the CNT have been modified by chemical binding of various

chemicals using these chemical functional groups.

Further, there have been reported that the functional group of CNT was substituted with –SH group by chemical manipulation and patterned on a gold surface using the technique of micro contact printing (Nan, X. *et al.*, *J. Colloid Interface Sci.*, 245:311-8, 2002) and that CNT was immobilized on surface in the form of a multilayered film using the electrostatic method (Rouse, J.H. *et al.*, *Nano Lett.*, 3:59-62, 2003). However, the former has disadvantages of the low CNT surface density and the weak binding power, and the latter also has a fatal disadvantage in that the patterning method for selective immobilization on the surface cannot be applied. Therefore, there is an urgent demand for developing a new type surface immobilizing method.

At the present, it has been known that the functions of 10 thousand genes among about 100 thousand human genes come out into the open and most of the genes directly relate to diseases. Also, medical materials over 95% which are developed or are being developing target protein because most of the diseases are caused in protein level not DNA level. Therefore, on the basis of data obtained by protein function analysis, network analysis and function analysis of bio-molecule interacting with specific protein and ligand, the detection technique of the protein-protein reaction and protein-ligand reaction is necessary to study treating and preventing the diseases which have been impossible to treat and prevent by the conventional method.

Recently, researches are being conducted to detect reactions by means of electrochemical changes of CNT attached biomaterials, using electrical properties, semiconductor properties and structural stable properties of CNT(Dai, H. *et al.*, *ACC. Chem. Res.*, 35:1035-44, 2002; Sotiropoulou, S. *et al.*, *Anal. Bioanal. Chem.*, 375:103-5, 2003; Erlanger, B.F. *et al.*, *Nano Lett.*, 1:465-7, 2001; Azamian, B.R. *et al.*, *JACS*, 124:12664-5, 2002).

A representative example of a protein-ligand reaction is an avidin-biotin reaction. It is about to form a channel on a substrate, which had been treated with a polymer, using CNT and measure the binding activity of streptoavidin

according to an electrochemical method (Star, A. *et al.*, *Nano Lett.*, 3:459-63, 2003).

The reasons that CNT attracts public attention as a biochip are as followings: Firstly, it needs no labeling; secondly, it has high sensitivity to signal change; and thirdly, it is capable of reacting in an aqueous solution without modification of a protein. Combination of a new nanomaterial and a biological system will create important applied technologies in respective fields of disease diagnosis (hereditary diseases), proteomics and nanobiotechnology.

In order to develop a rapider and cheaper biochip, many researches have been conducted on technologies of DNA hybridization detection. Various labeling technologies for detecting DNA hybridization have been developed. At the present, the fluorescent materials are generally used for labeling. A single DNA strand is fixed, which is capable of detecting complementary DNA and the single DNA strand detects the complementary DNA in solution, and a signal converter change a DNA hybridization signal to a specific signal which can be analyzed.

An effective surface treatment capable of increasing hybridization efficiency and simultaneously, removing the background from non-specific binding is required to detect the DNA hybridization effectively using the DNA chip. Many researches have been conducted to prepare a surface-treated DNA chip platform (*Anal. Biochem.*, 266:23-30, 1999; *Nuc. Acid. Res.*, 29:107, 2001).

Many applications with CNT in the bioengineering field have recently been appeared, such as glucose biochip, detecting protein, detecting a certain DNA sequence and the like (*Anal. Bioanal. Chem.,* 375:103-5, 2003; *Proc. Natl. Acad. Sci. USA,* 100:4984-9, *Anal. Bioanal. Chem.,* 375:287-93, 2003). Screening bio-molecules from multilayer based on CNT can increase the amount of immobilized bio-substances, such as DNAs and detecting sensitivity to the bio-substances, since the CNT has wide surface area and high electrical conductivity.

At the present time, the most universal method for detecting the reaction

result in a biochip is to use conventional fluorescent materials and isotopes (Toriba, A. *et al.*, *Biomed. Chromatography:BMC.*, 17:126-32, 2003; Raj, S.U. *et al.*, *Anal. Chim. Acta*, 484:1-14, 2003; Peggy, A.T. *et al.*, *J. Microbio. Meth.*, 53:221-33, 2003). However, as novel methods to easily and precisely measure an electrical or electrochemical signal are attempted, there are increased demand for CNT as a new material.

It has been reported that PNA (peptide nucleic acid: DNA mimic) is position-specifically fixed on a single walled CNT and the complementary binding to target DNA is detected (Williams, K.A. *et al.*, *Nature*, 420:761, 2001). Also, there have been an example, in which an oligonucleotide was fixed on a CNT array by an electrochemical method and DNA was detected by guanidine oxidation (Li, J. *et al.*, *Nano Lett.*, 3:597-602, 2003). However, these methods do not apply CNT in fabrication and development of biochips.

In recent, a high capacity biomolecule detection sensor using CNT was disclosed (WO 03/016901 A1). This patent relates to a multi-channel type biochip produced by arranging a plurality of CNTs on a substrate using a chemical linker and attaching various types of receptors. It has a disadvantage of relative weakness to environmental changes.

Meanwhile, the conventional DNA chip which is fixed an oligonucleotide or DNA on a amine treated surface has a disadvantage of difficulty in evenly attaching DNA with high density on the surface because the amine groups treated on the surface are not distributed evenly. Also, it has a disadvantage of producing many unnecessary signals when analyze the result of hybridization using fluorescent materials.

Therefore, the present inventors have fabricated a CNT-DNA chip by fixing CNT with high density on a surface having exposed amine groups by chemical bonding to form a high density CNT film having exposed carboxyl groups and have found unnecessary signals dramatically decreased and the desired fluorescent signals were detected with strong fluorescent intensity when analyze

the signals with fluorescent materials as a result of hybridization, and completed the present invention.

【TECHNICAL OBJECT OF THE INVENTION】

It is an object of the present invention to provide a CNT film comprising CNT fixed at a high density on a substrate by chemical bonding and having chemical functional groups exposed on its surface, a CNT-biochip comprising bio-receptors attached onto the surface of the CNT film.

It is another object of the present invention to provide a method for detecting various target biomaterials capable of binding to or reacting with a bio-receptor using the CNT-biochip.

It is a further object of the present invention to provide a CNT-DNA chip comprising DNA attached to CNT film at a high density on a substrate by chemical bonding, a method for detecting DNA hybridization comprising using the DNA chip.

【CONSTITUTION OF THE INVENTION】

To achieve the above object, the present invention provides a method for producing a high density CNT film having carboxyl groups exposed, which comprises the steps of: (a) reacting a substrate having amine groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amine group and the carboxyl group; (b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; and (c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT film having exposed carboxyl groups.

The present invention also provides a high density CNT film having exposed carboxyl groups which is prepared by the above-described method.

The present invention also provides a CNT-biochip comprising a bio-receptor fixed to the carboxyl group exposed on the CNT film by chemical or physicochemical bonding, in which the bio-receptors have a functional group capable of binding to the carboxyl group.

Also, the present invention provides a method for producing a high density CNT film comprising a chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen, exposed on its surface, which comprises the steps of (a) reacting a substrate having amine groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amine group and the carboxyl group; (b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; (c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT film having exposed carboxyl groups; and (d) modifying the high density CNT having exposed carboxyl groups with a chemical compound having both a functional group capable of binding to the carboxyl group and a chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen.

According to the present invention, the chemicals having both the functional group capable of binding to carboxyl group and the chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen include HN_2-R_1-NH_2, NN_2-R_2-SH, HN_2-R_3-OH or HN_2-R_4-CHO, in which R_1, R_2, R_3 and R_4 are independently a C_{1-20} saturated hydrocarbon, un-saturated hydrocarbon or

aromatic organic group.

The present invention also provides a high density CNT film which is prepared by the above-described method and has a chemical functional group exposed on its surface, in which the chemical functional group is selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen.

The present invention also provides a CNT-biochip comprising a bio-receptor fixed to a chemical functional group, selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen, exposed on the CNT film by chemical or physicochemical bonding, in which the bio-receptor has a functional group capable of binding to the chemical functional group.

Also, the present invention provides a method for detecting a target biomaterial capable of binding to or reacting with a bio-receptor comprising using the CNT-biochip according to the present invention.

Also, the present invention provides a CNT-DNA chip using DNA as a bio-receptor and a method for detecting DNA hybridization comprising using the CNT-DNA chip.

According to the present invention, the substrate may be selected from the group consisting of silicone, glass, melted silica, plastics and PDMS (polydimethylsiloxane), the substrate having exposed amine functional group may be prepared by treating amine alkyloxysilane.

According to the present invention, the chemical functional group capable of binding to carboxyl group is preferably amine group or hydroxyl group.

According to the present invention, the bio-receptor may be, for example, enzyme substrates, ligands, amino acids, peptides, proteins, nucleic acid (DNA, RNA), lipids, cofactors or carbohydrates, which have carboxyl group, amine group, hydroxyl group, aldehyde group, or thiol group.

According to the present invention, the target biomaterial may be a substance able to serve as a target reacting with or binding to the bio-receptor to be detected, including preferably proteins, nucleic acids, antibodies, enzymes, carbohydrates, lipids or other biomolecules derived from living bodies, more preferably nucleic acid or proteins.

The term "CNT-biochip" used herein inclusively refers to composites having a bio-receptor chemically or physicochemically bonded to a CNT chip and may be defined as biosensor comprising a bio-receptor attached to a high density CNT chip by chemical or physicochemical bonding.

According to the present invention, the CNT-biochip capable of detecting various types of target biomaterials directly or by an electrochemical signal is fabricated by repeatedly laminating CNT on a solid substrate coated with a chemical functional group (amine group) by chemical bonding to prepare a high surface density CNT film having exposed carboxyl groups and attaching a bio-receptor having a functional group (amine group, hydroxyl group, etc.) capable of chemically or physicochemically reacting with the carboxyl group.

Also, the biochip is fabricated by forming the high density CNT film having exposed amine groups by modification of carboxyl group exposed on CNT film surface and chemically or physicochemically attaching a bio-receptor having a carboxyl group or aldehyde group capable of chemically reacting with the amine group.

Meanwhile, in order to attach a bio-receptor without having a functional group capable of binding to the carboxyl group or amine group, the CNT film having the exposed carboxyl group is modified with a chemical compound having both a chemical functional group capable of binding to the carboxyl group and a chemical functional group capable of binding to the functional group of the target bio-receptor. Therefore, nearly all bio-receptors can be chemically or physicochemically attached to the high density CNT film.

For example, in order to attach a bio-receptor having thiol group, a CNT

film is firstly modified with a chemical having both a chemical functional group capable of binding to the carboxyl group and the thiol functional group so that the thiol functional group is exposed on the surface of the CNT film. Then, a bio-receptor having a thiol group is attached to the CNT film by S-S bond formation.

Also, according to the present invention, it is possible to attach bio-receptor with high density because the surface of high density CNT film has abundant chemical functional groups, and detect strong fluorescent signals because unnecessary signals decreased when detect the result of the hybridization using CNT-DNA chip with fluorescent materials.

The present invention will hereinafter be described in further detail by examples. However, it is to be understood that these examples can be modified into other various forms, and the scope of the present invention is not intended to be limited to such examples. Such examples are given to more fully describe the present invention for a person skilled in the art.

Particularly, the following Examples just instantiate DNA as a bio-receptor, it will however be obvious to a person skilled in the art to fabricate a CNT-biochip by fixing a bio-receptor to CNT film, wherein the bio-receptor is an enzyme substrate, a ligand, an amino acid, a peptide, a protein, RNA, PNA, lipid, a cofactor or carbohydrate having chemical functional group such as carboxyl group, amine group, hydroxyl group, aldehyde group, thiol group.

Example 1: Preparation of CNT having exposed carboxyl groups

The CNT which can be used in the present invention is not particularly limited and may be commercially available products or prepared by a conventional method. Pure CNT should be carboxylated at its surface and/or both ends to be used in the present invention.

The CNT having exposed carboxyl groups was refluxed in a sonicator containing a strong acid (a mixture of nitric acid and sulfuric acid for 24 hours

and filtered through a 0.2μm filter. The residue was dipped in a condensed acid, heated to reflux at 90 for 45 hours and centrifuged. The supernatant was collected, filtered through a 0.1μm filter, and dried. The dried CNT having exposed carboxyl groups was dispersed in distilled water or an organic solvent, filtered through a 0.1μm filter to obtain CNT with a predetermined size.

Example 2: Preparation of a substrate having exposed amine group

In the present invention, the substrate having exposed amine group was prepared by fixing amine alkyloxysilane on a substrate such as silicone, glass, melted silica, plastics, PDMS (polydimethylsiloxane). However, commercially available substrates which had been surface-treated with amine can also be used.

Example 3: Preparation of a high density CNT film having carboxyl group exposed on its surface

The CNT having exposed carboxyl groups, prepared in Example 1, was reacted with the substrate having exposed amine groups, prepared in Example 2 to form a CNT single layer on the substrate by amide bond formation between the carboxyl group and the amine group (FIG. 1(a)).

Then, the CNT attached to the substrate by amide bond was reacted with a diamine type organic compound having double amine functional groups while CNT having exposed carboxyl groups was reacted with amine groups at the other side of the diamine type organic compound to form another CNT layer by amide bond (FIG. 1(b)).

Next, the chemical reaction between the CNT having exposed carboxyl groups and the diamine type organic compound was repeated to prepare a high density CNT film comprising the CNT layer and the organic amine layer alternately laminated for n times and having carboxyl groups exposed on its surface (FIG. 1(c)).

The diamine type organic compound which can be used in the present invention includes compounds having a formula of HN_2-R_1-NH_2, in which R_1 is

C_{1-20} saturated hydrocarbons un-saturated hydrocarbons or aromatic organic group.

To accelerate the above amide bond, HAMDU(*O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorphosphate), DCC(1,3-dicyclohexyl carbodiimide), HAPyU(*O*-(7-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)uronium hexafluorphosphate), HATU(*O*-(7-azabenzotriazol-1-yl)-1,1:3,3-tetra methyluronium hexafluorphosphate), HBMDU(*O*-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorphosphate), or HBTU(*O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) is preferably used as a coupling agent, and DIEA(diisopropylethylamine), TMP(2,4,6-trimethylpyridine), or NMI(*N*-methylimidazole) is preferably used as a co-coupling agent.

Also, in the case of use water as solvent, EDC(1-ethyl-3-(3-dimethylamini-propyl) arbodiimide hydrochloride) is preferably used as a coupling agent, and NHS(*N*-hydroxysuccinimide) or NHSS(N-hydroxysulfosuccinimide) is preferably used as a co-coupling agent.

In this Example, HATU was used as a coupling agent and DIEA was used as a co-coupling agent.

The coupling agent participates in the formation of the amide bond (-CONH-) between the -COOH functional group and the $-NH_2$ functional group, and the base and the co-coupling agent act to increase the efficiency when the coupling agent forms the amide bond.

Example 4: Fabrication of CNT film having exposed amine groups on its surface

The modification of CNT surface having exposed carboxyl groups may be modified by chemicals having both a chemical functional group (amine group, hydroxyl group, etc.) capable of reacting with the carboxyl group and a chemical

functional group (amine group, hydroxyl group, thiol group, aldehyde group, etc.) capable of binding to a functional group of a desired bio-receptor. The chemicals which can be used in such modification include HN_2-R_1-NH_2, NN_2-R_2-SH, HN_2-R_3-OH, HN_2-R_4-CHO and the like, in which R_1, R_2, R_3 and R_4 are independently a C_{1-20} saturated hydrocarbon, un-saturated hydrocarbon or aromatic organic group.

In this example, CNT film having a amine group exposed on its surface was fabricated by binding carboxyl group of CNT film having a carboxyl group exposed on its surface prepared by example 3 with amine group of chemical of HN_2-R_1-NH_2.

Example 5: Fabrication of a DNA chip comprising DNA fixed on a CNT film having exposed carboxyl groups

A DNA chip was fabricated by chemically attaching DNA to the CNT film having exposed carboxyl groups. A CNT-DNA chip was fabricated by attaching amine groups of DNA to the CNT film having exposed carboxyl groups, prepared in Example 3 (FIG. 2). In this Example, EDC was used as a coupling agent for the amide bond between the carboxyl group and the amino group and NHS was used as a co-coupling agent. Also, in this Example, a CNT-DNA chip was fabricated using oligonucleotide having the following SEQ ID NO: 1 having amine group as the terminal group.

SEQ ID NO: 1 : 5'-TGT GCC ACC TAC AAG CTG TG-3'

The existing of DNA to the CNT film was confirmed by XPS (X-ray photoelectron spectroscope) spectrum for phosphorous atom considering the fact that all DNAs have phosphate groups (FIG. 4). As shown in FIG. 3, phosphorus was detected in the XPS surface analysis and thus, it was confirmed that DNA was attached to the CNT surface.

Example 6: Fabrication of a DNA chip comprising DNA fixed on a CNT film having exposed amine groups

In this Example, a CNT-DNA chip was fabricated by attaching carboxyl groups of DNA to the CNT film having amine groups exposed on the surface, prepared in Example 4 (FIG. 3). In this Example, EDC was used as a coupling agent for the amide bond between the carboxyl group and the amino group and NHS was used as a co-coupling agent. Also, in this Example, a CNT-DNA chip was fabricated using oligonucleotide having the SEQ ID NO 1 having carboxyl group as the terminal group.

Example 7: Hybridization analysis using a DNA chip comprising DNA fixed on a CNT film having exposed carboxyl groups

The CNT-DNA chip prepared in Example 5 was placed in a hybridization chamber and a hybridization solution was drop with pipette. Then, a cover slide was placed thereon. Here, the hybridization solution was mixed with 32 μℓ of a solution containing an oligonucleotide of complementary sequence to a total volume of 40 μℓ at a final concentration 3XSSC (0.45M NaCl, 0.045M sodium citrate) and 0.3% SDS(sodium dodecyl sulfate). The complementary oligonucleotide sequence was the following SEQ ID NO 2 having FITC (fluorescein isothiocyanate) attached to its end.

SEQ ID NO 2: 5'- CAC AGC TTG TAG GTG GCA CA-3'

The solution was left at 100 for 2 minutes and centrifuged for 2 minutes at 12000rpm to remove non-specific binding of two oligonucleotide strands. In order to prevent the hybridization solution from being dried in the hybridization chamber, 30 μℓ of 3XSSC (0.45M NaCl, 0.045M sodium citrate) was placed in hollows at both sides of the chamber and the chamber was closed and hybridized for 10 hours at 55 in a incubator.

After 10 hours, the hybridization chamber was took out of the incubator and immersed in 2XSSC solution for 2 minutes, then immersed in solution of

0.1XSSC (0.015M NaCl, 0.0015M sodium citrate) and 0.1% SDS for 5 minutes and finally was immersed in 0.1XSCC for 5 minutes. In order to remove remaining solution on DNA chip, the chip was placed in a centrifuge and centrifuged for 5 minutes at 600rpm.

The hybridization was detected through a fluorescent image using FITC labeled at the end of the oligonucleotide of the SEQ ID NO 2. The fluorescent image was obtained using ScanArray 5000 (Packard BioScience, BioChip Tecnologies LLC) confocal microscope and the QuantArray Microarray Analysis Software (FIG. 5).

FIG. 5(a) shows a fluorescent image of the high density CNT film, before binding of DNA, in FIG. 5(b), (1) shows the result of the fluorescence detection upon hybridization with complementary DNA, after binding DNA having amine group as the terminal group thereto to the above CNT film, (2) shows the result of the fluorescence detection upon hybridization with non-complementary DNA. It was confirmed that the fluorescence was clear and even when the oligonucleotide having the sequence complementary to the CNT film was hybridized ((1) of FIG. 5(b)). However, in the CNT film without having the oligonucleotide fixed thereon (FIG. 5(a)) and in the CNT film hybridized with the oligonucleotide having the non-complementary sequence ((2) of FIG. 5(b)), no fluorescence was observed. From these results, it was confirmed that the non-specific reaction almost never occurred.

Example 8. Hybridization analysis using a DNA chip comprising DNA fixed on a CNT film modified with amine groups

A hybridization was performed following the process as described in Example 7 using the CNT-DNA chip prepared in Example 6 (FIG. 6). FIG. 6(a) is the result of the fluorescent detection upon hybridization with complementary DNA and (b) is the result of the fluorescent detection upon hybridization with non-complementary DNA. As shown in FIG. 6, as a result of hybridization of the oligonucleotide having the non-complementary sequence with the

oligonucleotide having the complementary sequence, it was possible to certainly distinguish between the hybridized sample and the non-hybridized sample.

【EFFECT OF THE INVENTION】

As described above, the present invention provides a high density CNT film having exposed chemical functional groups on the surface which is produced by repeatedly fixing CNT having carboxyl groups exposed on a substrate having exposed amine groups by amide bond and a biochip comprising a bio-receptor chemically or physicochemically bonded to the surface of the CNT film.

According to the present invention, it is possible to fabricate CNT-biochip by chemically or physicochemically attaching various bio-receptors to a CNT film having exposed carboxyl groups or a CNT film having the exposed carboxyl groups modified by various chemical functional groups. Also, it is possible to fabricate a CNT-biochip comprising bio-receptors attached evenly at a high density on a surface of a CNT film where chemical functional groups are abundant and present evenly.

Particularly, upon fluorescent measurement of DNA hybridization using the CNT-DNA chip according to the present invention, it is possible to reduce unnecessary signals, thereby producing excellent results. The CNT-DNA chip is useful for genotyping, mutation detection, pathogen identification and the like.

【THE CLAIMS】

【CLAIM 1】

A method for producing a high density CNT film having a carboxyl group exposed on its surface, which comprises the steps of:

(a) reacting a substrate having amine groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amine group and the carboxyl group;

(b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon; and

(c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT film having exposed carboxyl groups.

【CLAIM 2】

A method for producing a high density CNT film having a chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen, exposed on its surface, which comprises the steps of:

(a) reacting a substrate having amine groups exposed on the surface with CNT having exposed carboxyl groups to form a CNT single layer on the substrate surface by amide bond formation between the amine group and the carboxyl group;

(b) reacting the CNT single layer with a diamine type organic compound to form an organic amine layer on the CNT single layer and reacting the

organic amine with the CNT having exposed carboxyl groups to laminate a CNT layer thereon;

(c) repeating the step (b) n times to form CNT layers and organic amine layers alternately laminated for n times, thereby forming a high density CNT film having exposed carboxyl groups; and

(d) modifying the high density CNT film having a carboxyl group exposed on its surface with a chemical compound having both a functional group capable of binding to the carboxyl group and a chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen.

【CLAIM 3】

The method according to claim 1 or 2, wherein the substrate is selected from the group consisting of silicone, glass, melted silica, plastics and PDMS.

【CLAIM 4】

The method according to claim 1 or 2, wherein the substrate having exposed amine functional groups on its surface is prepared by treating the substrate with amine alkyloxysilane.

【CLAIM 5】

The method according to claim 1 or 2, wherein the chemical functional group capable of binding to carboxyl group is amine group or hydroxyl group.

【CLAIM 6】

The method according to claim 2, wherein the chemical compound having

both the functional group capable of binding to carboxyl group and the chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen include HN_2-R_1-NH_2, NN_2-R_2-SH, HN_2-R_3-OH or HN_2-R_4-CHO (in which R_1, R_2, R_3 and R_4 are independently a C_{1-20} saturated hydrocarbon, unsaturated hydrocarbon or aromatic organic group).

【CLAIM 7】

A high density CNT film which is prepared by the method of claim 1 and has a carboxyl group exposed on its surface.

【CLAIM 8】

A high density CNT film which is prepared by the method of claim 2 and has a chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen, exposed on its surface.

【CLAIM 9】

A CNT-biochip which is comprising bio-receptors fixed to the carboxyl group exposed on the surface of CNT film of claim 7 by chemical or physicochemical bonding, in which the bio-receptors have a functional group capable of binding to the carboxyl group.

【CLAIM 10】

The CNT-biochip according to claim 9, wherein the bio-receptor is an enzyme substrate, a ligand, an amino acid, a peptide, a protein, nucleic acid, lipid,

a cofactor or carbohydrate.

【CLAIM 11】

The CNT-biochip according to claim 10, wherein the bio-receptor is a peptide, a protein or DNA.

【CLAIM 12】

The CNT-biochip according to claim 9, wherein the chemical functional group capable of binding to carboxyl group is amine group or hydroxyl group.

【CLAIM 13】

The CNT-DNA chip according to claim 9, wherein the bio-receptor is DNA.

【CLAIM 14】

A CNT-biochip which is comprising bio-receptors fixed to the chemical functional group selected from the group consisting of amine group, aldehyde group, hydroxyl group, thiol group and halogen exposed on the surface of CNT film of claim 8 by chemical or physicochemical bonding, in which the bio-receptors have a functional group capable of binding to the chemical functional group.

【CLAIM 15】

The CNT-biochip according to claim 14, wherein the bio-receptor is an enzyme substrate, a ligand, an amino acid, a peptide, a protein, nucleic acid, lipid, a cofactor or carbohydrate.

【CLAIM 16】

The CNT-biochip according to claim 15, wherein the bio-receptor is a peptide, a protein or DNA.

【CLAIM 17】

The CNT-DNA chip according to claim 14, wherein the bio-receptor is DNA.

【CLAIM 18】

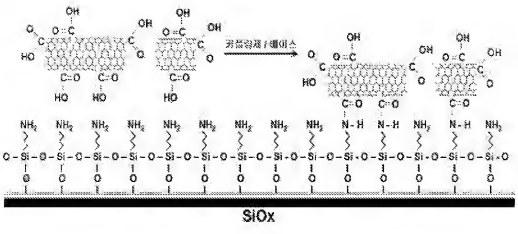
A method for detecting a target biomaterial capable of binding to or reacting with a bio-receptor using the CNT-biochip of one claim among the claims 9 to 12 and 14 to 16.

【CLAIM 19】

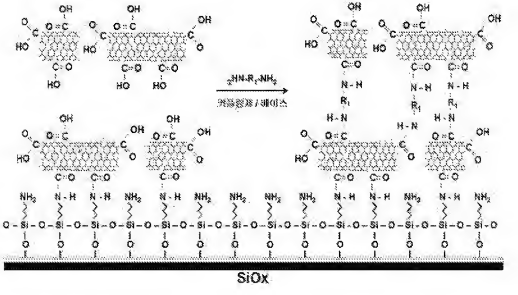
A method for detecting DNA hybridization comprising using the CNT-DNA chip of claim 13 or 17.

【DRAWINGS】

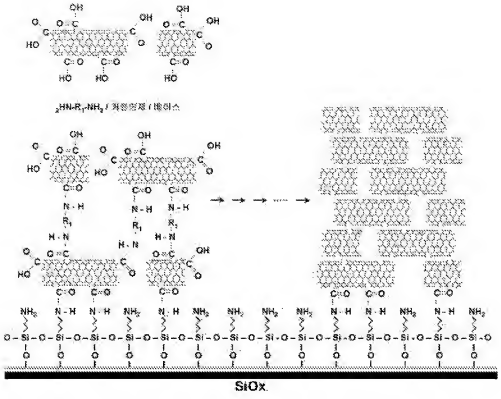
【FIG. 1a】



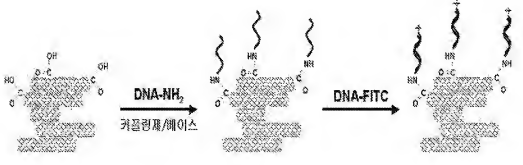
【FIG. 1b】



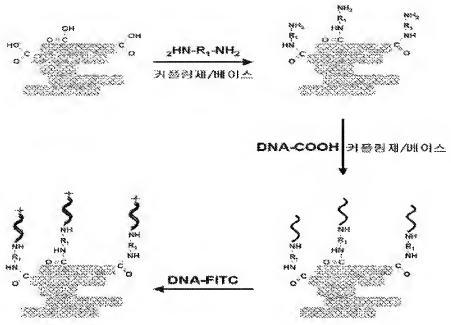
【FIG. 1c】



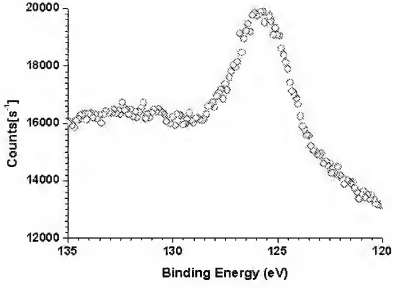
【FIG. 2】



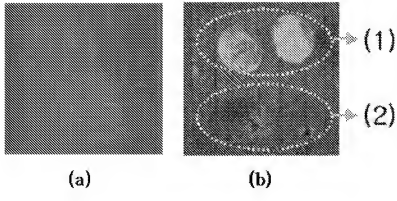
【FIG. 3】



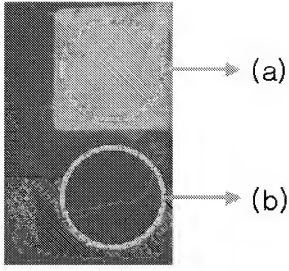
【FIG. 4】


20000
18000
16000
14000
12000
Counts[s⁻¹]
135
130
125
120
Binding Energy (eV)

【FIG. 5】


(1)
(2)
(a)
(b)

【FIG. 6】



【SEQUENCE LIST】

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